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Response to External Conditions
Studies upon a Fusarium

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RESPONSE TO EXTERNAL CONDITIONS: STUDIES UPON A FUSARIUM

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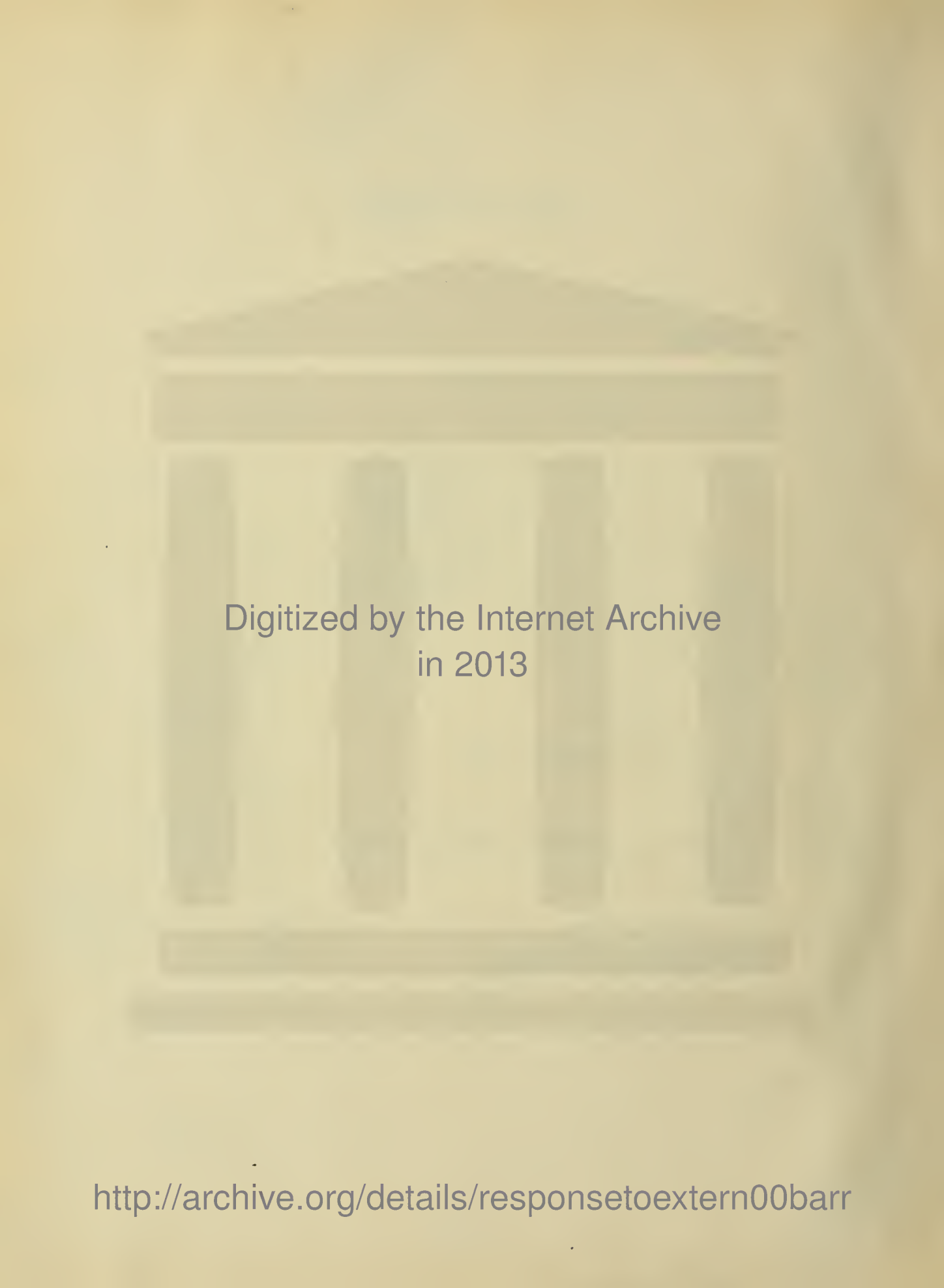

This thesis is based on investigations made in the laboratories of the botanical department of the University of Illinois, as a part of the required work preliminary to the granting of the degree of Master of Arts. The work has been carried on under the supervision of Professor T. J. Burrill, to whom I have been under obligations for suggestions and for help in the matter of literature on the subject.

INTRODUCTION

While engaged in a study of dry rot of corn during the fall and winter of 1905, a species of Fusarium was frequently met with which when grown in pure culture was seen to possess some rather peculiar characteristics. It proved to be a rapid grower and capable of developing an abundance of mycelium on many kinds of natural media. For a time it was grown beside and compared with two other species of Fusarium also taken from decaying corn. A study of the fungus was decided upon and the results are included in this paper.

METHODS

In carrying on experiments reported herein strict regard was paid to all bacteriological methods concerned. All cultures used were descendants from a single original microconidium. A few of the spores were germinated in a growing cell and by means of a sterile needle the small colony produced by one conidium was lifted out and placed in a culture tube, where it soon produced a mass of mycelium. The purity of the cultures could never be doubted and

the line of descent was likewise unquestionable. Transfers were made in a quiet atmosphere and in as nearly a sterile situation as could be obtained. Sterilization was done with an Arnold's steam sterilizer and an accurately tested autoclav. All solutions were made up with chemically pure substances where necessary, and titrations were made with standardized solutions. Especial care was taken that all tubes were thoroughly cleaned before using.

DESCRIPTION OF THE FUNGUS

Mycelium. As the fungus grows on the infected corn it may or may not produce a large mass of compact white mycelium, depending on the conditions during its period of active development. Specimens of both types of growth have been found. Almost pure cultures have been secured by inoculating a culture tube with a bit of the much crumbled interior of an infected grain. The mycelium wends its way among the starch grains apparently partially digesting them.

On a plate of agar, asparagin glucose agar for instance, the growth was very rapid-- a centrally located colony becoming two and one-half inches in diameter in less than four days. The formation of the colony was brought about by the radial outward growth of the newly formed filaments from the point of inoculation, and with the longitudinal growth considerable branching took place. The margin of this colony was approximately circular, as is usually the case on agar poured plates. The amount of aerial mycelium varies with the medium and the amount of moisture present. On rice, for instance, the aerial mycelium attains a height of 10 to 20 m.m. in a few days, while on tapioca it never gets more than 1 to 3 m.m. high.

The mycelium is made up of large and small filaments interwoven and frequently much coalesced, the latter depending largely on the kind of medium used, and when occurring to any considerable extent the growth has a stringy or ropy appearance. The larger filaments are a sign of vigorous,active growth and vary in size from 6 to 10 microns. In a poured agar plate under a 1/6 inch objective the growth at the tip of a large hypha was measured and its onward movement could be detected. In such a culture, if the medium is favorable to growth, the white aerial mycelium soon appears, usually about the center of the colony first, and then gradually progresses toward the periphery of the dish, rarely keeping pace with the growth of the imbedded portion. The small or conidia-bearing filaments make their appearance sooner or later, depending on conditions which are favorable or averse to good vegetative development. For instance, lack of moisture aids in bringing about the formation of small filaments and a production of conidia. These small strands are formed as branches of the larger ones and may result as the gradual decrease in size of the latter, or arise abruptly from them (Plate 3, Fig.1). The small filaments vary in size from 2 to 4 microns in diameter and hence are small only in comparison with the larger ones.

The mycelium varies not a little in form, size, and appearance on different media, a subject more fully treated under the head of Media. The mycelium in a young culture is usually filled with granular protoplasm, becoming during the rapid growing period much vacuolated, and in certain old cultures quite well filled with very refractive fat bodies. Apparently with the ceasing of the vegetative activities, especially on very starchy media, the minute fatty drops which give the granular or turbid appearance to the protoplasm

during the active growing period, collect into large, strongly refringent drops and occupy the greater part of the cells.

Microconidia. The method of the formation of microconidia was best studied in Van Tieghem cells in which various culture materials were used. Prune juice (50%) proved to be very favorable for such a culture liquid. It was just acid enough (+ 15) to have a retarding effect soon after some mycelium was formed, as a result of which conidia were soon produced. As seen under the microscope the microconidia are colorless, obovate to pyriform, and vary in size, sometimes considerably, with the media used. When first formed the protoplasmic content is uniform or granular, but may soon become vacuolated. The size varies from 7 1/2 - 9 x 6 - 8 microns.

Micioconidia are produced terminally on simple or much branched sporophores (Plate 1, Figs.1,6,7,& 10). The end of a terminal or hypha, more frequently a lateral branch, is cut off from the remaining portion by a rather narrow constriction. The newly formed microconidium is pushed aside by the growing hypha on the tip of which a slight constriction soon begins to appear. The growth of the new microconidium takes place by a gradual swelling of this small constricted portion, until, when the growth is complete, it is cut off. Several observations made on growing cell cultures showed that the average interval of time between the cutting off of one and the cutting of a second microconidium was about one hour. The process continues until, frequently, a number of conidia are produced from one branch.

Germination tests of the microconidia made in several kinds of liquid media showed that there was quite a variation in the time required for germination and for different individuals. One Van Tieghem cell was made from each culture fluid and inoculated with

microconidia from a rice culture. The cells were kept at room temperature 22° to 25°C. and were examined, at first, at frequent intervals.

Microconidia sown in Uschinsky's fluid (1) began to germinate in 5 hours, and in 9 hours practically all were pushing out germ tubes, many of which were quite long. At the end of 22 hours all microconidia, with very few exceptions, had germinated and some germ tubes had formed several branches. In 48 hours quite a mat of mycelium had been formed and both microconidia and macroconidia were being produced.

In Raulin's (2) fluid the microconidia began to germinate after 15 hours, and within 22 hours about one-half had pushed out very short germ tubes. At the end of 48 hours practically all microconidia had germinated but the much branched germ tubes showed very little increase in growth and no new conidia were being produced.

In standard beef bouillon, germination began within 3 hours, and in 5 hours one-third of the microconidia had germinated. At the end of 9 hours all microconidia had sent out germ tubes, many of which were quite long. In 22 hours the filaments or germ tubes had become much branched and had formed a tangled mass of mycelium. In 48 hours no conidia had been produced.

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(1) Uschinsky's fluid: water,250 c.cm.; glycerine,7.5 c.c.; ammonium lactate,1.5 c.cm.; sodium chloride .75 grm.; sodium asparaginate,.75 grm.; dipotassium phosphate,.5 grm.; calcium chloride,.025 grm.; and magnesium sulphate .05 grm.

(2) Raulin's fluid: water,250 c.cm.; cane sugar, granulated, 17.6 grms.; tartaric acid,.66 grm.; ammonium nitrate,.66 grm.; ammonium phosphate,.10 grm.; magnesium carbonate,.066 grm.; ammonium sulphate,.042 grm.; zinc sulphate, .012 grm.; ferrous sulphate, .12; and potassium silicate, .012 grm.

In a solution containing 2% Witte's peptone, and 1% glycerine, germination began in 3 hours and in 5 hours a goodly number had produced germ tubes of considerable length. The swollen microconidia had a peculiar warty appearance. In 9 hours practically all microconidia had germinated and in 22 hours the germ tubes had become quite long, with few branches. At the end of 48 hours the growth appeared weak and a few microconidia and macroconidia were being produced.

In distilled water probably one-fourth of the microconidia had germinated in 5 hours. In 9 hours one-half had germinated, but the germ tubes were short and grew slowly. In 22 hours all microconidia had germinated and many of the short germ tubes were cutting off small microconidia (Plate 2, Fig.1). In 48 hours there was practically no increase in the growth of the mycelium; but the number of small microconidia had increased considerably.

In prune juice (50%) germination began in 5 to 6 hours, and in 12 hours quite a number of microconidia had sent out vigorous germ tubes. After 24 hours practically all microconidia had germinated and the germ tubes were forming a mat of mycelium (Plate I, Fig.1).

In 100% prune juice germination had scarcely begun in 20 hours but in 36 hours most of the microconidia had germinated. After germination was well under way growth was more rapid than in the 50% prune juice. After the culture had partially dried down a little distilled water was added with the result that in a day or so both microconidia and macroconidia were being produced in considerable numbers.

Macroconidia. In so far as specimens have been examined, the occurrence of this form of conidium on corn is rare. In fact an abundance of them was found but once and then on a diseased embryo

ear of corn which, after being kept in the laboratory for several months, had developed this form of fruit. In artificial cultures, many of which were made, macroconidia occurred only a few times in anything like large numbers. Since this kind of spore is the one upon which the form-genus Fusarium is commonly based it was necessary that it should be found occurring naturally in order that the species could be properly classified. Not until the work herein described had neared completion was the mature macroconidia found on the specimen mentioned above.

The macroconidia vary very much in form and size, ranging as to the latter in nature from 10 - 25 x 4 - 8 microns. The average size, however, of mature three-celled conidia was found to be 18 - 22 x 5 - 6 microns. While some are straight, they are usually slightly curved and slightly constricted at the septa (Plate III, Fig.5). In artificial cultures they are usually rounded at the distal end and taper toward the bluntly acute proximal end (Plate I, Fig.9). The number of septa vary from 1 - 4, the usual number being 2 - 3. On the natural specimen there are about as many that are acute at both ends as there are of the form mentioned.

Macroconidia are formed, so far as a study in liquid media has revealed the process, in very much the same way, and sometimes on the same hyphae on which the microconidia are borne. This was observed in both prune juice and Uschinsky's fluid (Plate I, Fig.6). So far as observations in this connection could be made the macroconidia producing hyphae were little, if any, different from those which produced the microconidia. In both cases there is usually a swollen portion in the middle of the branch and a slight constriction at the point of attachment to the mycelial filament.

In tube cultures, both microconidia and macroconidia, but more

particularly the latter, fell off soon after being formed, or on being disturbed, and unless examinations were made during the active periods, they escaped notice.

Germination tests were made in connection with those made with microconidia. Both kinds were in the same cells, with fewer macroconidia than microconidia.

In Uschnisky's fluid a few macroconidia had germinated in 5 hours and nearly all had begun to swell. In 9 hours all had germinated.

In Raulin's fluid little germination had taken place in 22 hours, but in 48 hours nearly all macroconidia had germinated.

In Standard beef bouillon germination began in 3 hours. In 5 hours two-thirds of the macroconidia had germinated. In 22 hours the mycelium from the two kinds of conidia had formed quite a dense mat.

In a solution of 2% Witte's peptone, 1% dextrose, 1% maltose, and 1% mannite, one-fifth of the macroconidia had germinated in 3 hours. Fully one-half had germinated in 5 hours and all in 9 hours.

In a solution containing Witte's peptone 2% and glycerine 1%, germination began in 3 hours. About one-fourth had germinated in 5 hours and one-half in 9 hours. In 22 hours all had germinated.

In distilled water about the same condition existed as with microconidia.

In prune juice rather old macroconidia began to germinate in 20 hours, and in 48 hours quite a film of mycelium had formed on the cover glass.

In all solutions except distilled water and Raulin's fluid the

germ tubes grew and branched rapidly, forming within 48 hours considerable mycelium. No difference could be detected between the germ tubes and mycelium originating from the two kinds of conidia. Germination of the macrospores took place at either or both ends, or at one of the middle cells, rarely at all three places.

Chlamydosporoids. This term is used for a structure which resembles very much a true chlamydospore in its early stages of development. When first discovered in a test tube containing soaked corn, its resemblance to a young sporangium of a mucor caused the culture to be set aside as impure. Later, however, pure cultures made from descendants of a single microconidium produced the same peculiar structure, and a careful examination showed it to be a part of the fungus under consideration. Whereas the true Chlamydospores of the other Fusaria studied, three species, are usually borne in an intercalary manner this chlamydosporoid is more frequently produced terminally. They are borne on a rather long, sparingly septate hyphalbranch of the aerial mycelium. Usually these branches are parts of the larger type of mycelial filaments, however they may be produced on those of either size. As a rule they are spherical in shape, thin walled, and filled with granular protoplasm. In some favorable cultures they become quite large (Plate II, Fig.3). They are rather evanescent in character, rarely being found in cultures in which the mycelium has ceased to be active. Their formation seems to bear a rather close relationship to the moisture content and composition of the culture medium used. They were rarely found submerged in liquid cultures, but when they were, they were borne both in an intercalary and terminal manner. Such was the case in a culture liquid consisting of 2% peptone, 1% dextrose, 1% maltose, and 1% mannite. Chlamydosporoids were produced

on several solid media, such as boiled rice, boiled cracked corn, boiled corn husks, raw potato (intercalary), boiled potato, boiled sweet potato, potato agar, beef agar, and a few others. Certain acid, and some of the weaker alkaline, rice cultures were also favorable media. Whereas the true chlamydospores of Fusarium are ordinarily produced in culture media and under conditions quite severe for the production of microconidia and macroconidia, the chlamydosporoid is found, as a rule, in the more vigorous cultures and on media quite well adapted for good vegetative growth.

Coiled Hyphae. This form of hypha usually appears in cultures which are favorable to the production of the chlamydosporoids, in fact, the two usually accompany each other. This structure varies considerably in shape and size, at times resembling the chlamydosporoid itself (Plate II, Fig.4,c). A filament of the larger mycelium coils or folds up at the end into a spherical or irregular oblong shaped mass, without destroying the outline of the filament (Plate II, Fig.4,a,b,d). The protoplasm is more frequently vacuolate when the structure has become of some age. A few cases were observed in which a chlamydosporoid had developed on a coiled hypha (Plate II, Fig.4, 2). The function of these rather peculiar structures was not determined.

GROWTH ON NATURAL MEDIA

With the hope of getting the fungus to form perithecia, or its sexual fruiting stage, a large number of different culture media were used, and under various conditions.

There are only a few cases on record of inducing the formation of perithecia from the conidial fructification of any Hypocreaceaus

fungus. According to Smith ('99), Brefeld, and Von Tavel do not record any, and only three such cases are mentioned by them for the whole group of acomycetes. Klebs has found the perithecial stage for Eurotium repens, and in 1895 Hugo Gluck ('95) recorded the same for Fusarium aquaeductuum. These facts lead to the belief that if the proper culture conditions could be ascertained many of the now so-called imperfect fungi could be induced to form the perithecial stage. However, with the use of a large number of various kinds of both natural and synthetic media all attempts failed in securing perithecia from this species.

On boiled corn husks, still partially green, growth was good, moderately dense, and extended well to the bottom of the tube, white above with a tinge of pink to red below. Some few chlamydosporoids, coiled hyphae, and many microconidia were produced. Seven weeks after inoculation some of the larger mycelial filaments were still in an active condition.

Growth on boiled corn was excellent, extending 15 to 25 m.m. above the surface in a few days. In 6 to 7 weeks the growth had taken on a felty appearance with a cream color above and dark pink to yellowish brown in the substrata. The pink color appeared within three days. Microconidia became numerous with a drying of the medium. A few each of macroconidia, chlamydosporoids, and coiled hyphae were produced.

Boiled rice proved to be a very good medium. The growth of the fungus was rapid, reaching a height of 12 to 15 m.m. in a few days. In 3 days tinges of light salmon were present, becoming more intense with age, frequently showing some red to purple. Within 6 days microconidia, macroconidia, and coiled hyphae were found. After 7 weeks there was very little activity and the mycelial threads

contained many large refractive oil globules.

On boiled potato the growth was soon compact and dense, but never attained a height of more than 4 to 6 m.m. After a few days there developed a slight tinge of light salmon which soon disappeared. At the end of 7 weeks the color was white with a little golden yellow at the upper portion. Microconidia were produced in abundance. No macroconidia, and but a few chlamydosporoids were found.

On raw potato there was a slight growth after a few weeks. The scattered mycelium was yellowish brown in appearance and bore a good many poorly developed chlamydosporoids, mostly of the intercalary type.

On sweet potato, which proved to be a very good medium, the fungus grew to be rather dense and 15 to 20 m.m. high. The color became light salmon to salmon in a few days, gradually increasing to pink, and even to red in the tube grown in the dark. Microconidia and chlamydosporoids were produced in fairly large numbers, the latter being few in number after 6 to 7 weeks.

On boiled barley the growth proceeded rapidly, attaining a height of 3 to 6 m.m. in 4 days, and filling the interstices with a rather sparse growth of mycelium. In 7 days there were a few patches of pink color, and in 17 days it had developed, in some places, to a light brown. Microconidia, macroconidia and coiled hyphae were produced.

On boiled tapioca the growth was very sparse, rarely ever attaining over 2 or 3 m.m. in height. After 7 weeks there were a few microconidia found among rather small filaments of mycelium. Most of the mycelial filaments were characterized by the presence of large oil globules. The growth of the fungus on boiled banana was rather distinctive in appearance. For a few days there was apparent-

ly a very poor growth, but at the end of a week the mycelium had grown from the slant opposite to the point of inoculation. In two weeks there was a fair growth of a dirty white, wet-like mycelium having a ropy appearance, surrounding the slant. The growth was somewhat better in the dark. Microconidia only were found.

On boiled prunes in 7 weeks the growth was sparse and weak in appearance. An examination at the end of this time showed only a few microconidia.

In 4 days the growth on boiled white beans was pretty good. The mycelium extended some distance into the substratum and a few m.m. into the air. A strong odor of hydrogen sulphide which was being given off at this time became less strong after a week, and at the end of three weeks could scarcely be detected. No colors other than cream and light drab were produced. All forms of conidia were found in small numbers.

On potato agar the growth was rather weak. The mycelium attained a height of 3 to 4 m.m. in a week and penetrated the substratum to about the same depth. In less than two weeks large numbers of microconidia and a few each of macroconidia and chlamydosporoids were being produced. After 9 weeks the color was still white.

The growth on peptonized potato agar was slightly more dense than on the ordinary potato agar, otherwise it was about the same.

In 4 days the growth on beef agar was 2 to 3 m.m. high in places and had penetrated the substratum fully as much. With age there was a production of a few each of microconidia and chlamydosporoids. The color remained white.

The growth on litmus-lactose-agar in 4 days was sparse and 2 to 3 m.m. high. The substratum showed a tinge of blue color.

With age the mycelium extended deeper into the medium, but never attained a greater height than that mentioned. In less than three weeks the medium was blue and the fungus had ceased to be active. Microconidia were produced in rather large numbers. Only a few chlamydosporoids were found.

On boiled turnip the growth was good, 4 to 5 m.m. high and dense. At the end of 4 weeks the fungus had ceased to be active. A few macroconidia and large numbers of microconidia were present at the end of 6 weeks. A few chlamydosporoids were produced.

The growth on boiled carrot was rapid, attaining a height of 5 m.m. and producing both microconidia and macroconidia in 3 days. In 8 days the growth had reached a height of 8 m.m. and the aerial portion had a tendency to be ropy. A few macroconidia, but no chlamydosporoids were found.

Boiled salsify produced a very good growth consisting of a compact mass of medium sized mycelial filaments. A few each of macroconidia and chlamydosporoids were found, while microconidia were numerous. With the exception of a tinge of golden yellow, the color was white.

Boiled parsnip produced a better growth than salsify. It attained a height of 12 to 15 m.m. in 3 days. The mycelial filaments were large, much branched, and filled with very granular protoplasm. Coiled hyphae were numerous and many of the large filaments were surrounded at irregular intervals with masses of substance,- probably secretion- which produced a peculiar knotted appearance (Plate II, Fig.5).

In 3 days the growth on boiled beet was 3 to 4 m.m. high, and white, tinged with light brown at the upper portion of the slant. With age the brown color increased in extent and the mycelium be-

came more or less ropy. Microconidia only were produced.

Boiled apple was a rather poor medium. Growth was very slow and remained pure white. The mycelial filaments were always very regular and sparingly branched. A very few microconidia were found.

In 3 days there was a good growth on boiled macaroni. A small area of bright red mycelium was present. Later the amount of color increased, but more salmon than red was present. At the end of 6 weeks the color had diminished considerably and a little brown was present. Microconidia were numerous.

On boiled cabbage the growth was poor. The mycelial filaments were small and with age became fused into ropes and bundles. Few microconidia were found and a rather strong, unpleasant odor was given off.

In 3 days the growth on boiled orange was 6 to 10 m.m. Later the white was tinged with cream and the protoplasm became quite vacuolate. The size and shape of the numerous microconidia varied considerably.

The growth on boiled bean stems was pretty fair, the maximum height being 4 to 5 m.m. In 3 days both microconidia and macroconidia were found inside the stems and some of each were germinating. Later the microconidia were produced in large numbers. The color remained white.

On boiled madiera bulb a fair growth of white mycelium was produced. A few microconidia were found.

On germinated corn the growth was good, only moderately dense, and white with a slight tinge of brown. A few macroconidia and many microconidia were produced.

Soaked corn produced a growth very similar to that on germinated corn. Growth was very good on cocoanut agar. The mycelium was

white, rather flocculent, and extended 20 to 25 m.m. up the sides of the tube in 3 days. Microconidia were produced in quite large numbers. A few coiled hyphae were found.

On cocoanut milk the growth covered the surface and extended well down into the liquid in 3 days. Later the aerial mycelium reached a height of several m.m. A light salmon color developed at the surface of the liquid. Microconidia and coiled hyphae were found on the aerial portion.

Uschinsky's fluid: In 5 days there was a light flocculent growth throughout the liquid, making it somewhat cloudy. Later a dense pellicle was formed on the surface from which aerial hyphae extended well up the sides of the tube. On these hyphae were borne tufts of both microconidia and macroconidia, some of which were germinating (Plate II, Fig.2). Growth increased for several days then gradually became inactive. A slight purplish color was produced in one tube on the under side of the surface growth.

Raulin's fluid: In 5 days there was no growth in one tube and only a small mass of mycelium in the bottom of the second. Later several small jelly-like colonies of mycelium developed. The mycelium was much branched and irregular in growth, contained granular protoplasm, and produced no conidia.

Beef bouillon: Growth was good and pretty rapid, forming a mass of mycelium on the surface 10 m.m. high in 5 days, and a sparse flocculent growth throughout the liquid. Both kinds of conidia were being produced. Later a good many chlamydosporoids were found (Plate I, Fig.10).

In a solution composed of Witte's peptone 2%, dextrose 1%, maltose 1%, and mannite 1%, the growth in 5 days was 5 m.m. high on the surface and extended all through it, giving it a very clouded

appearance. The mycelial filaments were irregular and very vacuolate. The aerial portion bore coiled hyphae and chlamydosporoids. The latter were also found in the liquid, as were many peculiar swellings of the mycelium (Plate II, Fig.6).

In a solution of Witte's peptone 2% and glycerine 1% a flocculent growth had developed throughout the liquid in 5 days. The protoplasm of the mycelial filaments was quite vocuolate. In 10 days microconidia, macroconidia, and a few coiled hyphae were being produced. In 3 weeks the growth had become 6 to 8 m.m. in height, the submerged portion was inactive and the protoplasm very granular.

THE EFFECT OF THE COMPOSITION AND REACTION OF MEDIA ON GROWTH AND COLOR PRODUCTION

Before giving the results of the experiments carried on relative to growth and color production a short space is devoted to a brief review of some of the more important literature on the subject.

Schacht ('63,p.446) found in the hollow spaces of decaying potatoes a mass of mycelial threads of Fusisporium solani Mart. which was characterized by the violet contents of the filaments and which gave a blue black appearance to the surrounding tissue. The filaments became rose red on the addition of sulphuric acid.

Smith ('99,p.23) said "On neutral or acid media in the presence of free oxygen and of starchy foods- e.g., potato, bread, rice, tapioca, wheat, hominy, cucumber agar, etc. this fungus develops in the substratum a series of the most brilliant colors, which are then absorbed by the hyphae. These hues include many shades of pink, red, purple, and violet, and in some of the substrata- e.g., bread or boiled rice-- are particularly brilliant, changing gradual-

ly from shades of purple and rose color into the deepest crimson (rose carthamine). This color is much brighter and purer than any I have been able to obtain with Went's Monascus purpureus. During the development of this pigment the substratum becomes intensely acid (mostly CO_2 but some lactic acid according to Mr. K.P.McElroy.) If, however, alkaline substances (caustic lime, carbonate of soda, etc.) be added to the substratum in advance, so as to neutralize the acid or acids as fast as formed, no color is developed, the fungus remaining snow white, as in the vessels of the melon plant. If less alkali be added, the colors appear gradually after a time, which is longer or shorter, according to the amount added."

Harz ('90) found a fungus in 1890 in a reservoir which grew in a liquid whose glycerine content was between 35 and 76.8% and whose ash content was between 2.4 and 3.1%. The temperature ranged from 32.8° to 34.6° C, and on the addition of more glycerine to 50° to 60°C. The fungus was called Physomyces heterosporus n.g. et n.sp., but is, according to E.Bessey, a species of Monascus. The color of the mycelium in the warm glycerine solution was dark brownish red, sometimes carmine to rose red. The color was not as intense in solid as in liquid media.

Smith and Swingle ('04) who worked on Fusarium oxysporum, found that the general effect of acids is to produce color, both lilac and pink. They found that if no acid be added to a rice tube and the tube be placed in the dark the growth remained almost white. In red light (behind a potassium bichromate screen) no color was produced, but behind a blue light (ammoniacal copper carbonate solution) the color appeared. The effect of alkali was to retard the formation of the pigments, and for a few days at least to retard the growth. After 17 days they found that the growth in the various

strengths of alkali had been greatly equalized.

Ostwalder ('04, p.212) in his study of Fusarium putrefaciens n. sp., found that there was a striking difference between this and Fusarium gemmiperda studied by Aderhold, in that the red coloring matter was not bound to the fat globules as Aderhold found, and was evidently not in the cell sap. From experiments made he concluded that it was possible that the cell membrane contained the color. Many times the greenish yellow color of mycelial filaments was changed to red by boiling in water, which he believed was a proof that there existed a close relationship between the two colors.

In a paper published in 1904 ('04, p.274) Thomas Milburn says: "By an increase in osmotic pressure the fungus Hypocrea rufa finally ceases to produce a pigment, and conidia formation is retarded." He found also that the color of the conidia was determined by the reaction of the medium, acid reaction producing green spores, and alkaline reaction yellow ones. Richly nourished mycelium produced no fruit in the dark, but when well supplied with oxygen, or poor nourishment furnished, conidia were developed. Hypocrea gelatinosa gave similar results.

MM. Henri Coupin et Jean Friedel, (Compt rend. de l'Acad.d. sc.T. CXXXVIII. Paris 1904. p. 118), (according to Milburn) obtained the following results on Sterigmatocystis versicolor. They found

1. Upon slightly acid media there was produced a yellow pigment.
   " neutral media " " " an orange "
   " alkaline " " " " a red "
2. An alcoholic solution of pigment on addition of acid became yellow, on addition of alkali red.
3. In Raulin's solution color of spores was green.

3. In Raulin's solution without Mg. } Color of spores
on Potato } grayred.
On carrot }

"Various species of fungi, according to their conidia belonging to the genus Fusarium, produce in various substrata and under various conditions, red, violet, blue, orange and yellow pigments" is a statement made by E.Bessey ('04, p.332). He also states "that the red pigment produced by Neocosmospo ra and two species of Fusarium (designated a & b) separated from diseased Sesamum is an acid compound, soluble in alcohol and many other solutions. Its salts are mostly of a violet color and insoluble in the above solutions, and soluble only in the salts of some organic acid. The orange pigment formed by the same fungi under the influence of light is not a lipochrom. Its definite chemical nature could not be determined."

"The coloring matter of Fusarium culmorum has an acid, yellow, and an alkaline, violet, form. The acid form seems to be a weak organic acid and is only slightly soluble in alcohol or water; the alkaline form is soluble in alcoholic and aqueous solutions of alkalies." He found further that the red and violet pigments produced by both the Fusariumand Neocosmospora were not dependant on the composition of the culture media used. Colorless mycelium produced in an acid medium and transferred to a very weak alkaline one soon developed color; mycelium that had been produced in an alkaline culture and left there remained colorless. Strong acid checked the formation of color, as did an insufficient supply of oxygen, however, the fungi under favorable conditions were capable of growing anaerobically. Osmotic pressure above a certain stated degree, as extremes of high and low temperatures made pigment formation impossible. Certain poisonous substances checked the formation of the coloring matter entirely."

Pollock ('06) found in a study of a species of Fusarium collected on the cut ends of corn stubble, that under proper conditions for development a bright salmon pink color developed. Among the conditions necessary for its development are direct sunlight, in the absence of which only a pale cream color is produced, generally without a tinge of red. Moisture is also a factor in the development of color.

Description of experiments.

The results of culture experiments on natural media showed that there were several substances of a starchy nature which were well suited to both growth and pigment production. Ground corn, cracked barley, rice and some others answered very well, but rice being white after boiling, and not so compact as the others, was selected for most of the following experiments that were made on solid media, especially in reference to acid and alkaline reactions.

Alkali. To test the effect of various percents of alkalies on growth and color production there was instituted a series of 9 tubes each containing 1 gram of rice and 4 c.cm. of various strengths of sodium hydroxide solutions making the media of the following strengths:(1) check no alkali, - 6 1/4,- 12 1/2,- 25,- 50,- 100, -150,-200,-250. The stock solution of sodium hydroxide used was $\frac{n}{4}$,or -250. The tubes were sterilized 30 minutes in the steamer, and on the following day 10 minutes in the autoclave at 10 lbs. pressure. After sterilization there was a gradation in the color of rice ranging from white to orange with the increased strength of the

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(1) Fuller's scale was used to reckon strengths of acids and alkalies.

alkali. In the higher strengths the individuality of the rice grains were destroyed, a jelly-like mass resulting. This was particularly noticeable in the -200 and -250 tubes. The tubes were inoculated with mycelium and conidia from an old rice culture and incubated 24 hours at 29°C. In 3 days there was a fair growth of white mycelium in the check, -6 1/4, - 12 1/2, and -25 tubes and a slight growth in -50. The other tubes showed no growth. In 6 days the growth had increased considerably and up to -50 about the same amount with more color in -12 1/2 and -25, there being in these considerable pink to red surrounding many of the rice grains. Tube -100 showed a slight, wet-like surface growth of dense mycelium. No growth had developed in the other tubes. In 7 weeks there was some increase in growth in all tubes and -150 had produced a slight amount of mycelium. The colors, especially in the substrata, had increased in intensity until those in tubes -6 1/4, - 12 1/2, and -25 were partially dark purple to bay. No growth took place on the -200 tube. The contents of the tubes were titrated with the following results:

	As made up	After 7 weeks
No.1.	Check	+2 3/5
No.2.	- 6 1/4	+3
No.3.	- 12 1/2	+2
No.4.	- 25	+1
No.5.	- 50	- 2 2/5
No.6.	-100	- 6 1/4
No.7.	-150	- 4
No.8.	-200	- 20
No.9.	-250 no growth	- 50

A series of 6 tubes was next instituted, which contained rice and alkali in the same proportions as those used in the series above up to the seventh tube. The alkali used in this case was sodium carbonate. In this series there was about the same gradation of growth and color after 7 weeks as was found with the sodium hydroxide series, while the maximum color appeared in the substrata of tube - 6 1/4. Aside from the check the amount of growth was not quite so good as that in the corresponding tubes of the sodium hydroxide series. No growth took place in the - 100 tube. A titration of the contents gave the following results:

Original	After 7 weeks
No.1. Check	+4
No.2. - 6 1/4	+4 4/5
No.3. - 12 1/2	+2 1/5
No.4. - 25	- 2 2/5
No.5. - 50	- 12
No.6. - 100	- 56

The results of these two experiments shown in Table I were at first somewhat surprising in that the fungus was capable of maintaining a growth, even for a time, on a - 200 medium, and that, contrary to the results obtained by Smith ('99), Bessey ('04), and others, color production could be induced in alkaline media of the strengths used. The titrations showed a very great reduction in the alkalinity, too much apparently to be caused by the acidifying effect of the fungus. Under conditions of high temperature it is known that alkalies have a reducing effect on the hexoses and it was suggested that herein lay the solution of the difficulty.

To make further tests two sets of a third series of 7 rice tubes were made, one set to be titrated immediately after steriliza-

Table I. – Comparison of Growth in Alkaline Media.

Kind of medium	Alkalinity.	Height of Growth	Density of growth	Kind of Spores.			Color.
		Mm.					
Rice + NaOH	– 6 1/4	4–5	Dense	Microconidia	– – – – –	– – – – – – – –	White, salmon, orange.
Rice + NaOH	– 12 1/2	4–5	"	"	– – – – – –	– – – – – – – –	White, salmon, golde yellow and orange.
Rice + NaOH	– 25	3–4	"	"	Few Macroconidia	– – – – – – –	White, salmon, pink, orange and red.
Rice + NaOH	– 50	3–4	"	"	" "	– – – – – –	white, salmon, light pink and reddish purple
Rice + NaOH	– 100	1–3	Sparse	"	– – – – – –	– – – – – –	White, slight tinge of salmon
Rice + NaOH	– 150	1	Very sparse	– – – – – –	– – – – –	– – – – –	White.
Rice + NaOH	– 200	– – –		– – – – – –	– – – – –	– – – – –	– – – – – – – – – – – –
Rice + NaOH	– 250	– – – –	No growth	– – – – – –	– – – – –	– – – – – –	– – – – – – – – –
Rice. Check.	– 0	6–8	Dense	Microconidia	Few Macroconidia	Chlamydosporoids	White, salmon, pink to light brown.
Rice + Na_2CO_3	– 6 1/4	3–5	Dense	Microconidia	Macroconidia	– – – – –	White, salmon, golden yellow and orange.
Rice + Na_2CO_3	– 12 1/2	4–5	"	"	"	– – – – – –	White, salmon and orange.
Rice + Na_2CO_3	– 25	3–5	"	"	Few "	– – – – – – –	White, a little salmon, and considerable golden yellow.
Rice + Na_2CO_3	– 50	3–4	Moderately dense	"	– – – – – –	– – – – – –	Mostly White.
Rice + Na_2CO_3	– 100	– –	No growth	– – – – – –	– – – – – –	– – – – – –	– – – – – – – – –
Rice. Check.	0	8–9	Dense	Microconidia	Few Macroconidia	– – – – – –	White, salmon, and golden yellow.

tion. Each tube contained one gram of rice and 5 c.cm. of a dilute solution of potassium hydroxide of the strength to give the required reaction to the medium. The relative reactions before and after sterilization were as follows:

Before Sterilization	After Sterilization
1. - 2	1. +2
2. - 5	2. +1
3. -10	3. 0
4. -20	4. - 3
5. -30	5. - 4
6. -50	6. - 8
7. -about 0	7. about 0

The remaining tubes were inoculated with Fusarium mycelium and spores and kept at room temperature.

In 4 days there was a better gradation of growth, decreasing with the increased amount of alkali, than at any time after, a salmon color had appeared in tube No.3, and light salmon in No.2. There was a sparse growth in No.4, and a very poor growth in No.6. The following day a tinge of salmon appeared in tube No.1, and microconidia were rather abundant. The growth in No.3 had attained a height of 2 to 4 m.m., and the intensity of the salmon color had increased. Microconidia were being produced in goodly numbers and a few macroconidia were found. In No.3 the growth was almost as good as in No.2, with light salmon appearing. Microconidia present. Growth in No.6 was fair, with very little salmon color appearing. As growth continued the substrata became yellow to drab, while the mycelium continued to show deeper tinges of salmon. On the 13th day tube No.5 had considerable bright red color along the margin of the slant, with a light tinge of salmon in the aerial mycelium.

Tube No.6 had a poorer growth but more red color than No.5. The condition at the end of 24 days was as follows: The check showed considerable light pink to salmon in the mycelium with little color in the substratum. Tube No.1 had less color in the mycelium with some yellow, drab, and brown in the substratum. Tube Nos. 4,5, and 6 showed beautiful shades of pink, red, purple and dark purple in the mycelium along the margin of the slant and deep into the medium which was mostly dark purple in color.

A titration of the contents of the above tubes after the last examination gave the following results:

Before Inoculation		After 24 days	
1.	2	1.	+12
2.	1	2.	+10
3.	0	3.	+ 8
4.	- 3	4.	+7.5
5.	- 4	5.	+8
6.	- 8	6.	+6
7.	about 0	7.	+7

It will be seen from these reactions that the fungus produced considerable acid, more than was produced in the sodium hydroxide and sodium carbonate tubes. The relative reaction of the tubes in the inoculated series after 24 days compares very well with that of the series titrated soon after sterilization. From the results of the above experiments it seems quite probable that the amount and intensity of color produced is influenced considerably by the products formed as a result of the action of the alkali on the rice under the influence of high temperatures. It is necessary, however, that, after this reaction has taken place, the reaction should be either very slightly acid or slightly alkaline, preferably the latter.

In order to test more fully this action of alkalies a series of 6 tubes containing a 5% glucose solution of the reactions 0, -10, -20, -30, -50, and -100 was made up and sterilized in the autoclave at 110 C for 20 minutes. On removal from the autoclave the solutions showed a regular gradation of color ranging from colorless through yellow to brown, with the increased amount of alkali. Titrations of these solutions showed a very slight acid reaction in the 0 and more acid in all others except -50 and -100 which had a slight alkaline reaction, the -100 testing −3. It was very evident that one of the hexoses at least, glucose, could not be used in the presence of alkali when high temperatures were necessary for sterilization.

Carbohydrates. The following experiment was made in order to determine which carbohydrate among the few chosen was best suited for inducing growth and color production of the fungus. The following carbohydrates were used:

Monosaccharides	Polysaccharides
Glucose	Soluble starch
Galactose	Starch
	Cellulose (Filter paper)

Disaccharides

Maltose

Cane sugar

The solutions contained 5% of the carbohydrate and .5% Knop's solution. Each tube contained 10 c.cm. of the culture fluid.

Knop's Solution

$Ca(NO_3)_2$	4 grms.
Mg_2SO_4	1 grm.
K_2NO_3	1 "

K_3PO_4	1 grm.
Water	100 c.cm.

The tubes were sterilized on three successive days for 10 minutes in the steamer at 100°C. and after inoculation from a macaroni culture were placed in the incubator at 29°C, where they remained for three days. Subsequently they were kept at room temperature. At the end of four days there was a pretty fair growth on the surface of the liquid in the glucose, galactose, and cane sugar tubes extending a few m.m. into the solution and in the glucose culture 12 to 15 m.m. up the sides of the tube. The maltose solution was very much clouded by a flocculent growth of mycelium, while the growth in the soluble starch, starch, and cellulose cultures was slight. No color other than white had yet appeared, except a tinge of pink in the glucose tube, which on the following day had developed into a much deeper tinge. In 9 days the growth had increased considerably in all tubes except the cellulose, and a pink color was appearing in the cane sugar solution, while that of the glucose had a light brick color. It was 19 days before a very noticeable growth appeared on the surface of the maltose culture, the solution was still clouded with the jelly-like mass of mycelium. At this time a dense white precipitate filled most of the soluble starch solution at the top of which was a small mass of golden to brown colored mycelium. Subsequent to the 19th day there was loss of color in some of the tubes, a slight increase in growth of the aerial mycelium in the maltose tube, a liquefaction of the starch paste, and an increase in the amount of precipitate in the soluble starch solutions, in one case of the latter it became solid. More color was produced in the glucose and cane sugar tubes while the maltose solution developed the best growth. Glucose and cane sugar

were chosen as the carbohydrate basis of the liquid culture solutions to be used in the following experiments as best suited, all things considered, for producing color and growth of the fungus.

Experiments showed that either asparagin or peptone could serve as a source of nitrogen in the culture media, but because of the lighter color of the asparagin solutions and their being less apt to contain alkaline by-products common to peptone solutions, the former was more frequently used.

The first solution made up for testing the effect of alkalies on the fungus was made up as follows:

Cane sugar	5 grams
Asparagin	.1%
Knop's solution	.5%
Water to make	100 c.cm.

Eleven tubes each containing 10 c.cm. of the solution were made with the following strengths of alkalinity: Check 0, -.1, -.5, -1, -2 1/2, -5, -10, -20, -30, -40, and -50. The strength of KOH used in making up the solutions was $\frac{n}{}$ or -250. After sterilization duplicate tubes of -.5, -2 1/2, -10, -30, and -50 were titrated and found to be as follows:

As made up		After Sterilization
No.2.	-.5	- .66
No.4	- 2 1/2	- 2 1/2
No.6	- 10	- 8
No.8	- 30	- 27 1/2
No.10	- 50	- 45

After 4 weeks the differences in the amount of growth was quite apparent decreasing with the increase in amount of alkali present. The check and the tubes with the weaker alkaline reactions produced a salmon color on the aerial portion. At the end of 7 weeks the

growth in all tubes except the -45 had become practically equalized. All had surface growths with a little aerial mycelium and a dark cream to light salmon color, more in the check. The -45 tube had a rather dense growth in the upper portion of the liquid but no aerial growth. The contents of the tubes were retitrated with the following result:

Before Inoculation		After 7 weeks	
1.	- .1	1.	+3
2.	- .66	2.	+2.5
3.	- 1	3.	+3
4.	-2.5	4.	+2.2
5.	- 5	5.	+4.5
6.	- 8	6.	+5
7.	-20	7.	+3
8.	-27.5	8.	+2.5
9.	-30	9.	+3.5
10.	-45	10.	+4
11.	Check	11.	+4

Uninoculated checks of Nos.2,4,6,8,10 and 11 on titration after 7 weeks showed the following reactions: +.2, +2.5, -7, -18, -23, and +4.

A second alkaline series using the same nutrient solution as above was tried with sodium carbonate as the alkali, the -250 strength being used as a stock solution. Eleven tubes were made up having the same reactions as in series one. Duplicate tubes of -.5, -2.5, -10, -30, and -50 on titration after sterilization gave the following result:

As made up		After Sterilization	
No.2.	- .5	-	.66
No.4.	-2.5	-	2.5
No.6.	-10	-	6.3
No.8	-30	-	30
No.10.	-50	-	50

In 7 days some growth had developed in all tubes except -40 and -50. There was a thin surface growth with a slight tinge of pink in all tubes below the -20 strength. In 18 days growth had increased in all tubes, considerably in the weaker strengths and only slightly in the three or four stronger ones. There was a gradation of pink color beginning with the check and decreasing with the increase of alkali. At the end of 7 weeks the amount of growth in all tubes below -20 had become almost equalized in amount and the pink color at first apparent had changed to a dark cream and light salmon. The growth in the -20 tube was rather dense and filled the liquid producing a thin pellicle on the surface. No color was produced.

The -30 tube contained a fair growth which filled the lower three-fourths of the liquid. The upper surface was tinged with salmon, a rare thing in submerged growths. The growths in the -40 and -50 tubes was rather dense and occupied the lower one-half of the liquid. The contents of the tubes were titrated with the following results:

Before Inoculation		After 7 weeks	
1.	- .1	1.	+3
2.	- .66	2.	+4
3.	- 1	3.	+2.5
4.	- 2.5	4.	+3
5.	- 5	5.	+2

6.	- 6.3	6.	+4.5
7.	-20	7.	+5
8.	-30	8.	-20
9.	-40	9.	-32
10.	-50	10.	-40
11.	-0 Check	11.	+5 check

Uninoculated checks of Nos. 2,4,6,8,10, and 11 on titration after 7 weeks showed the following reactions: .3, -2, -5, -32, -50, and .3.

Acids. In testing the effect of acid media on growth and color production the following acids were used:

Formic acid, Acetic acid, Butyric acid } Acetic series. Monobasic.

Oxalic acid — Oxalic series. Dibasic.

Malic, Tartaric, Citric } Dibasic, Tribasic } Hydroxy acids.

Lactic acid

Hydrochloric acid

Nitric acid

Sulphuric acid

The first experiment consisted of a series of 12 tubes of rice, one for each of the above named acids and one for a check. All but the last were made up to a +25 reaction. Each tube contained 2 grams of rice and 10 c.c. of the dilute acid. The tubes containing the rice and 9 c.c. of distilled water were sterilized for 20 minutes in the autoclave at 110°C in a slanting position. After cooling 1 c.c. of the proper strength of acid was added and the tubes again sterilized for a few minutes in the autoclave which had previously been raised to 100°C. The presence of some of the acids during a

very long heating prevented the rice grains from puffing up and eventually made a mass having a more or less jelly-like consistency which was not so favorable for studying the growing fungus. The results of this experiment are seen in the accompanying table. At the strength used the fungus was unable to grow in the presence of formic, acetic, butyric, oxalic, and nitric acids, and very little with hydrochloric and sulphuric acids. The growth for several days was much slower than on the check, but in three weeks most of the tubes which showed any growth had a very dense mass of mycelium with tinges of salmon, pink, yellow, and one or two a little orange. At the end of 8 weeks the amount of growth had increased very little but the intensity of the various shades of color had increased quite noticeably. Growth was best on tartaric, citric, and lactic acids, not quite so good on the latter as on the two former. Malic produced a rather dense growth, a little less in amount however than lactic acid. The check had a very dense growth.

A second series of rice tubes contained the same acids of the following strengths:

Formic acid	+12 1/2	Tartaric acid	+50
Acetic "	+12 1/2	Citric "	+50
Butyric "	+12 1/2	Lactic "	+50
Oxalic "	+12 1/2	Nitric "	+12 1/2
Malic "	+50	Hydrochloric "	+12 1/2
		Sulphuric acid	+12 1/2

After inoculation the tubes were incubated 40 hours at 39°C, after which they were kept at room temperature in diffused light.

In 6 weeks there was a very dense growth on the formic and acetic media with considerable salmon to red color, particularly on the latter. The malic, tartaric, and citric tubes had a moderately

dense growth tinged with the above mentioned colors. The lactic acid tube had a very dense growth while that produced in the inorganic tubes was sparse with just as much, if not slightly more, color. There was no development in the acetic and butyric acid tubes. See Table II.

A third series of acid tubes was instituted with rice made acid with tartaric acid. Six tubes of the following reactions were used: Check 0, +5, +10, +15, +20, and +25. Duplicates of the check and +25 were made and after sterilization titrated. The +25 showed a +24 reaction and the check +.5. After inoculation the tubes were incubated at 29° C for 24 hours and then left at room temperature in diffused light. For 7 to 10 days the amount of growth was inversely proportional to the strength of acid, but in 2 weeks to 20 days this difference was less apparent, the growths in the various tubes having become more or less equalized. In the cultures having a greater reaction than +5 the fungus did not readily penetrate the substratum. The most color was produced in the check, +5, and +10 tubes. The remaining tubes, however, developed a little bright yellow, golden yellow and red.

The next and fourth acid series was made with liquid media. The solution was made up as follows:

Glucose 3 grms.
Asparagin .1 grm.
Knop's solution .2%
Water to 100 c.cm.

The series consisted of 27 tubes having the following acids and reactions:

1. Formic, +12 1/2, +25
2. Acetic, +12 1/2, +25
7. Lactic, +50, +75, +100
8. Citric, +50, +75, +100

Table II. – Comparison of Growth in Acid Media.

Kind of medium	Acidity of medium	Height of growth	Density of growth	Kind of spores			Color.
	/	Mm.					
Rice + Formic acid.	+25	- - -	No growth	- - - - - - -	- - - - - - -	- - - - - - -	- - - - - - - - - - - -
Rice + Acetic "	+25	- - -	" "	- - - - - - -	- - - - - - -	- - - - - - -	- - - - - - - - - - - -
Rice + Butyric "	+25	- - -	" "	- - - - - - -	- - - - - - -	- - - - - - -	- - - - - - - - - - - -
Rice + Oxalic "	+25	- - -	" "	- - - - - - -	- - - - - - -	- - - - - - -	- - - - - - - - - - - -
Rice + Malic "	+25	4–6	Rather dense	Microconidia	- - - - - - -	- - - - - - -	White, salmon, golden yellow to light brown.
Rice + Tartaric "	+25	10–15	Dense	"	- - - - - - -	Chlamydosporoids	White, salmon, pink and a tinge of bay.
Rice + Citric "	+25	6–8	Dense	"	Macroconidia	"	White, ochraceous, pink to red.
Rice + Lactic "	+25	6–8	Rather Dense	"	"	- - - - - - -	White, salmon, little pink to bay.
Rice + Hydrochloric "	+25	/ /	" "	- - - - - - -	- - - - - - -	- - - - - - -	Ochraceous, tinge of pink.
Rice + Nitric "	+25	- -	No growth	- - - - - - -	- - - - - - -	- - - - - - -	- - - - - - - - - - - -
Rice + Sulphuric "	+25	/	Dense	- - - - - - -	- - - - - - -	- - - - - - -	Light orange.
Rice. Check	·	10–12	"	Microconidia	Macroconidia	Chlamydosporoids	White, salmon, pink and a little brick color.
Rice + Formic acid	+12½	4–6	Very dense	Microconidia	- - - - - - -	- - - - - - -	White, salmon, pink, pale brick and drab.
Rice + Acetic "	+12½	4–6	" "	"	- - - - - - -	Chlamydosporoids	White, salmon, bright red to brick red.
Rice + Butyric "	+12½	- - -	No growth -	- - - - - - -	- - - - - - -	- - - - - - -	- - - - - - - - - - - -
Rice + Oxalic "	+12½	- - -	" "	- - - - - - -	- - - - - - -	- - - - - - -	- - - - - - - - - - - -
Rice + Malic "	+50	3–5	Mod. dense	Microconidia	- - - - - - -	- - - - - - -	White, salmon, brick red to brown.
Rice + Tartaric "	+50	4–6	" "	"	Macroconidia	- - - - - - -	" " " " "
Rice + Citric "	+50	3–5	" "	"	- - - - - - -	Chlamydosporoids	White, cream to pink, tinge of brown.
Rice + Lactic "	+50	6–8	Dense	"	- - - - - - -	- - - - - - -	White, golden yellow, and a little pink and brown.
Rice + Hydrochloric "	+12½	2–9	Rather dense	"	- - - - - - -	- - - - - - -	White, salmon, golden yellow and pale brick red.
Rice + Nitric "	+12½	3–5	" "	"	- - - - - - -	- - - - - - -	White, light salmon, orange to brown.
Rice + Sulphuric "	+12½	2–4	Mod. "	"	- - - - - - -	- - - - - - -	" " "
Rice. Check		8–10	Dense	"	Macroconidia	Chlamydosporoids	White, salmon, pink, and a little brown.

3. Butyric, +3 1/8, +6 1/4
4. Oxalic, +3 1/8, +6 1/4
5. Malic, +50, +75
6. Tartaric, +50, +75
9. Hydrochloric +25, +50, +75
10. Nitric, +25, +50, +75
11. Sulphuric, +25, +50, +75
12. Check in tube
13. Check in flask.

In sterilizing the autoclave was heated up to 100° C, then the tubes put in and, with very little steam escaping, kept at 104° to 105° C for 30 minutes. The tubes were inoculated with mycelium from a boiled potato culture 5 weeks old and were kept at room temperature.

In 9 days no growth had appeared in most of the tubes. A few, however, showed a fair growth. Check 10 to 14 m.m. high above the surface of the liquid; butyric +3 1/8 had a suspended mass of mycelium at the bottom of the liquid about 1/2 inch in diameter; oxalic +3 1/8 about the same; oxalic +6 1/4 about half as much; malic +50 a growth in the bottom of the tube about the size of a pea; malic +75, growth 2/3 size of +50; tartaric +100, a very slight growth; citric +50, growth consists of a mass 1/2 inch in diameter at the bottom of the tube; check in the flask had a very good growth covering the surface of the liquid.

The acidity of a number of the tubes was changed as follows:

Formic +25 changed to +3 1/8
" +12 1/2 accidentally destroyed
Acetic +12 1/2 changed to +3 1/8
" +25 changed to +6 1/4
Butyric +3 1/8 left +3 1/8
" +6 1/4 changed to +1 1/2
Oxalic +3 1/8 left +3 1/8
" 6 1/4 to +1 1/2
Malic +50 left +50

Malic +75 to +25

Tartaric +50 left +50

" +75 to +12 1/2

" +100 to +25

Lactic +50 left +50

" +75 to +12 1/2

" +100 to +25

Citric +50 left +50

" +75 to +12 1/2

" +100 to +25

Hydrochloric +25 left +25

" +50 to +6 1/4

" +75 to +12 1/2

Nitric +25 to +12 1/2

" +50 to +6 1/4

" +75 accidentally destroyed

Sulphuric +25 left +25

" +50 to +6 1/4

" +75 to +12 1/2

The reaction of some of the tubes was tested.

Formic +3 1/8 was found to be +3 3/4

Butyric +3 1/8 " " " " +4 1/2

Oxalic +1 1/2 " " " " +4 1/2

Oxalic +3 1/8 " " " " +15

Malic +50 " " " " +65

Sulphuric +6 1/4 " " " " +12 1/2

Hydrochloric +6 1/4 " " " " +6 1/4

Nitric +6-1/4 " " " " +12 1/2

Sulphuric +25 " " " " +25

Hydrochloric +25 " " " " +25

All tubes whose acidity had been changed and those which had showed no sign of growth were reinoculated and placed in diffused light at room temperature. After 5 weeks the following condition was found. Check, very good growth both above and below the surface, white above, golden yellow below. A salmon color which had previously appeared had become less apparent. Formic +3 1/8, a white flocculent growth throughout the liquid; acetic +3 1/8, growth at the surface 2 m.m. in thickness, dark cream in color; acetic +6 1/4, growth at the surface dense , 4 m.m. in thickness, dark cream in color; butyric +1 1/2, surface growth wet-like and 3-4 m.m. in thickness, considerable salmon; butyric +3 1/8, or+4 1/2, good submerged growth, white; oxalic +1 1/2, good submerged growth and 2 m.m. high on surface, a tinge of pink color; oxalic +3 1/8, growth all submerged, not quite so good as +1 1/2; malic +25, much better growth than the other tubes of the malic series, growth 15 m.m. above the surface of the liquid with a reddish brown color at the lower portion; malic +50 tube produced a light salmon color. In the tartaric series the better growth was produced in the +25 tube consisting mostly of surface growth 2-3 m.m. in thickness. The color produced was light salmon. The submerged growth in the +50 tube was dense and made up of many small colonies. In the lactic series the +25 tube showed the best growth. A dense mass of golden yellow to salmon mycelium covered the surface; the liquid was tinged a light amber and contained very little submerged growth. The +12 1/2 tube produced a growth somewhat poorer than the +25 tube and with less color. The lower half of the liquid in the +50 tube was filled with a rather dense mass of mycelium. No color. No growth was produced in the +25 tube of the hydrochloric acid series, but a pretty good development took place in the +6 1/4 and +12 1/2 tubes with

more salmon color in the former. No growth developed in either tube of the nitric acid series. In the sulphuric acid series the most growth developed in the +6 1/4 (rather +12 1/2) tube. A moderately dense mass filled the lower half of the liquid. No growth appeared in the +25 tube while the +12 1/2 culture developed 20 to 30 very small colonies of mycelium at the bottom of the liquid. No color developed in this series. The check in the flask produced a very good growth and a bright salmon color in 2 weeks. The color gradually faded away with age.

Judging from the results of the above acid media experiments it seems quite apparent that some acids are much more injurious to this fungus than others. The most injurious are butyric, formic, and acetic acids, members of the acetic series. Those of the hydroxy acid group, malic, tartaric, and citric, with lactic are the most favorable. The strength of the acids of the latter group most favorable for growth and color production was for the liquid media used about +25 while for the rice cultures it was somewhat lower. The amount of color produced in the liquid cultures was usually less than that on rice and not so intense, ranging more frequently from light cream to salmon. As a rule the inorganic acids were very injurious, not so much so as the members of the acetic series but considerably more so than the hydroxy acids used.

GROWTH IN THE ABSENCE OF FREE OXYGEN

From the result of the experiment made the fungus is not a strict aerobe. The experiment consisted of ten fermentation tubes containing the following liquid culture media:

No. 1. two tubes of Uschinsky's fluid.

No. 2. two tubes of Raulin's fluid.

No. 3. two tubes of Beef bouillon.

No. 4. two tubes of a solution of 2% peptone, 1% dextrose, 1% maltose and 1% mannite.

No. 5. two tubes of a solution containing 2% peptone, 1% glycerine.

These tubes were inoculated with small pieces of conidia-bearing mycelium which was putpast the curve of tube, and in each case it ascended to the top of the closed end but in a few hours fell to the bottom.

In 5 days growth in Uschinsky's fluid, No. 1, had proceeded into the open end of the tube and out of the liquid along the sides of the bulb. In 10 days the most of the inner surface of the bulbs was covered with a thin layer of mycelium and there was a slight flocculent growth in the closed ends reaching within one-third of the top.

The growth in No. 2 was for a number of days very sparse, in fact it never became very dense nor extended more than a few m.m. into the closed end.

In No. 3 there was a good growth in the open end in 5 days, and in 10 days some gas had been produced. The dense growth had sealed the open end completely. A slight tinge of salmon appeared later but gradually disappeared in a few days. No growth developed in the closed end of the tube.

In No. 4 growth was rapid, soon sealing up the open end of the tube. Some gas was produced, very probably CO_2 and on account of the tube being sealed was forced back into the closed end. After 2 weeks quite a bright red color was produced on the under surface of the growth. No growth developed in the closed end.

The growth in No. 5 was not so good as that in Nos. 3 and 4.

In three weeks both tubes were sealed by the growth and a slight tinge of very light salmon was apparent. No growth developed in the closed end.

EFFECT OF TEMPERATURE

The results of the three experiments made showed that the amount of growth and color production was quite largely influenced by variations of temperature. Since it was necessary that some of the tubes should be kept in the dark in order to get the effect of the higher degrees of temperature, none had access to light in the first two experiments.

Six rice tubes containing 2 grams of rice and 10 c.c. of distilled water each, were inoculated with Fusarium mycelium and two placed in each of the following temperatures: 22° to 24°C, 29° to 30°C, and 37°C. Before submitting the tubes to these various temperatures they were left at room temperature two days in which time the fungus formed a fair amount of white mycelium.

There was a rapid continuation of growth in the tubes kept at the two lower temperatures, but in those kept at 37°C a check in growth ensued and the growth that had previously formed became injured and finally died, as was evidenced by the fact that when brought later into the light at room temperature no growth developed. No color developed in these tubes.

On the 9th day after placing the tubes in the various temperatures, at which time they were photographed (Plate IV, 1) there was a very marked difference in the amount of color, particularly in the substrata. Very little color developed in the aerial mycelium of the tubes of either temperature. Light salmon to salmon

with a little yellow to golden brown prevailed in the room-temperature tubes, while that in the 30°C tubes consisted mostly of purple to dark purple and brown. After 2 weeks the tubes were removed from the incubators and all were submitted to the action of light at room-temperature. The colors became more intense and a salmon color soon appeared in the aerial mycelium. No change, as stated above, was apparent in the 37°C tubes.

In a similar experiment with cracked barley as the culture medium, the differences as found with the rice tubes were apparent but not so marked. The fungus was not entirely killed in the 37°C temperature for on removal from the incubator it soon began to grow and in a few days was little different from that in the other tubes.

In the third experiment made with rice tubes a low temperature was substituted for the 37°C one and another for light added. Eight tubes were made up with rice as in the first experiment and divided into four sets of two tubes each. After inoculation they were placed in the following temperatures: 13° to 15°C in a cold dark room, 23°C to 25°C in a cold incubator, 30°C, and 23° to 25°C room-temperature and in diffused light.

From the first more pink and red pigment was produced in the 29° to 30°C tubes while the rapidity of growth was little if any better than that in the medium temperature tubes. Growth at 13° to 15°C was very slow requiring 10 to 12 days to cover the surface of the slant. No color except a very little yellow in the substratum of one tube was produced. The most pigment was produced at 29° to 30°C in the substrata, very little developing in the aerial mycelium. At 23° to 25°C in the dark there were produced colors ranging from yellow to ochre, and a tinge of red. In the light at 23°C to 25°C a beautiful salmon color developed in the aerial mycelium,

while in the substratum there was little color aside from a few tinges of yellow, golden yellow, and after a few weeks a tinge of red.

EFFECT OF LIGHT

No separate experiments were carried on in regard to this point as opportunities for such observations presented themselves in other experiments. All observations made lead to the conclusion that light has a very favorable effect in the production of pigment in the aerial mycelium. The colors usually produced under such conditions range from salmon to pink, frequently however some red develops. Lack of time prevented conducting any experiments relative to the comparative effects on color production of the blue and red rays of the spectrum. One of the tubes used in the last mentioned temperature experiment and kept at 13° to 15° C was after 2 weeks development accidentally broken just below the plug. After lying in diffused light near a window for a few days the previously white mycelium had developed a beautiful light salmon color.

OSMOTIC PRESSURE

To test the effect of osmotic pressure on color production the following experiment was instituted: Eleven tubes of culture media were made up with varying percents of sodium chloride and inoculated with mycelium and spores of the fungus. The following strengths were used: 1%, 2%, 4%, 6%, 8%, 10%, 12%, 14%, 17%, 20%, and a check. The experiment was permitted to run 4 weeks, at the end of which time the results were as follows: No growth in the 12% solu-

tion and above, the check, 1%, 2%, 4%, and 6% cultures each had a fair amount of surface growth which extended some few m.m. into the liquid. The only aerial mycelium produced was in the check tubes. Each of the tubes showing surface growths had produced a light salmon color of about the same intensity, slightly darker in the checks, 1%, and 2% tubes. In the 8% tube the rather dense white growth filled the lower 2/3 of the tube while that of the 10% solution was less than half in amount. Apparently the osrotic pressure had a greater effect on growth than on color production although the latter showed a slight variation.

INFLUENCE OF OXYGEN (Air)

It must have been plainly evident from the above liquid experiments, that the color in any culture always appeared first, and usually only, on the aerial portion of the growth. In the submerged portion when any color did appear it was in tubes not sealed by a surface growth of mycelium. Cultures which had produced a rather abundant growth on rice tubes with tight fitting cotton plugs, on the removal of the plugs increased in color very soon. In a few cases in which the liquid below a surface mass of mycelium had evaporated or had been taken up by the fungus leaving a space filled by gas, new mycelium, was produced on the lower surface of the old, extended into this space, but failed to produce any color.

THE PIGMENT

Pigment was produced in the aerial mycelium and frequently in the filaments imbedded in solid media, such as rice, barley, corn

etc. It was particularly noticeable that the mycelial filaments lying in closest contact with the rice grains were more highly colored than those farther away. Old cultures mixed with water for titration purposes rarely produced any coloring of the water emphasizing the assumption that the pigment was not produced outside of the organism and subsequently reabsorbed. The shades of color most frequently met with were salmon, pink, red, purple and dark purple. Orange and yellow occurred less frequently.

The salmon colored mycelium after treatment with 2% nitric acid soon lost its color while weak solutions, 1%,of hydrochloric, acetic, oxalic, and sulphuric acids made very little apparent change even after several hours. On the addition of alkaline solutions such as ammonium hydroxide, ammonium carbonate, sodium hydroxide, sodium carbonate, and potassium carbonate the salmon color was readily changed to a more or less purple color.Ammonium hydroxide produced the darkest shade of purple. After acting one hour sulphuric ether had produced practically no change in the appearance of the specimen. These specimens thus treated with acids were washed in distilled water and subjected to the action of strong ammonium hydroxide. Almost immediately the specimens which had been treated with acids other than nitric and oxalic became more or less purple in color. The color failed to appear, even after several hours, in the specimens treated with the nitric and oxalic acids. Evidently the pigment was destroyed by their action. Ethyl alcohol had the same effect as alkalies but working somewhat more slowly. Ammonium carbonate was less effective than the other alkalies used.

The dark purple pigment which developed in several media particularly in rice at 30° C was easily changed by weak acids. Hydrochloric, sulphuric, nitric, acetic, and oxalic acids destroyed the

color of the specimen without giving any to the liquid. Ammonium hydroxide paled the purple color slightly while sodium hydroxide destroyed it entirely, giving the liquid a pale pink tinge. The color was likewise destroyed by ethyl alcohol and the liquid made a bright amber while the action of sulphuric ether, although slow, resulted in coloring the liquid pale yellow. After 24 hours the conditions just described had changed very little. The specimen which had been treated with sulphuric acid,after washing and treatment with ammonium hydroxide,changed to the dark purple color almost immediately. This was true with all specimens which had previously been treated with acids. On adding ammonium hydroxide to the alcohol containing the specimen treated, the color was not restored to the specimen but the amber color of the liquid was changed to pink. This pink color was not destroyed on the addition of nitric acid but did disappear on treatment with hydrochloric acid. Ammonium hydroxide failed to restore the original purple color to the specimen that had been treated with sodium hydroxide, this was also true of specimens treated with acetic acid. The purple color was not changed by chloroform.

Some salmon and blue colored mycelium produced on a cocoanut milk culture by another species of Fusarium also taken from rotting corn, when treated with acids and alkalies gave practically the same results as those mentioned above. The salmon corresponded to the salmon and the blue to the purple.

These rather incomplete results favor the supposition that in the case of this species of Fusarium, at least, the salmon and purple colors are not due to two separate pigments but to two forms of the same one. Bessey ('04) came to the same conclusion in reference to the red and blue colors found in his study of some Fusariarium species.

He considered the red the acid, and the blue the alkaline, form of the compound. Smith ('99 p.21) supposed that he had two distinct pigments which developed in cultures of the cow pea fungus. The results mentioned above seem to favor the first assumption, that by Bessey. The salmon color resembling an acid and the dark purple an alkaline form of the same pigment.

BEARING ON SYSTEMATIC RELATIONSHIPS

A thoughtful review of the variations in manner of growth, in size and shape of conidia, and in shades of color produced by the fungus, will, I think, result in the opinion that they are dependant rather on the physiological effect of the environmental conditions than on inherent characteristics of the organism. Changes in composition of media bring about changes in form and structure of the fungus, variations not greater than those frequently used, apparently, for separating species of this genus Fusarium. If the above ideas are correct, and the experiments and observations cited lead the writer to believe that they are approximately so, then such characters as color, shape of conidia etc. should not be used indiscriminately as specific characters.

The close relationships of many of the species of Fusarium in the light of cultural experiments recently made on a few species of the same genus, seem to be too close to require separation into distinct species. Environment plays a very important part in the development of many fungi and more attention to this fact would, no doubt, prevent much confusion. Specimens as found in nature are not always of a definite type and when there is doubt cultural methods should be resorted to.

The species of Fusarium used in these experiments appears to have not yet been named but may be described as follows: Sporodochium effuse, white; sporophores simple to much branched, erect, septate; microconidia obovate to pyriform, 7 1/2-9x6-8 microns; macroconidia fusoid, frequently more acute at the proximal end, straight or slightly arcuate, 2-4 septate, slightly constricted, 12-22x5-6 microns.

After examining the descriptions of all species of Fusarium to which any reference could be obtained, it was found that the description given above agrees more closely with that of F.Ricini than any other, not sufficiently close however to make that name applicable.

SUMMARY

(1) The foregoing described observations and experiments go to show that this species of Fusarium, not yet determined but probably new, produces,in pure culture, a large amount of mycelium in a comparatively short time; that this mycelium is sooner or later made up of large and small filaments, the latter of which arise under less favorable conditions, abruptly from, or as a gradual reduction of, the former, and produce the microconidia and macroconidia, rarely chlamydosporoids and coiled hyphae.

(2) The formation of conidia is influenced largely by external conditions, e.i., insufficient suitable nourishment, lack of moisture, high temperature, and the composition and reaction of the medium. The three former favor production of conidia while the two latter may be so controlled as to be either favorable or unfavorable. Extremes in acid and alkaline reactions retard conidia production as well as growth, but reactions of less degree which are

very unfavorable to development of mycelium favor an increased production of conidia. Macroconidia are rarely if ever produced so abundantly in culture as microconidia and in only a comparatively few media; in fact, their formation seems to be more dependant on conditions unfavorable for vegetative development than that of microconidia.

(3) The peculiar structures chlamydosporoids and coiled hyphae, resemble somewhat in their earlier stages of development the young sporangium of Mucor and the young ascogonium of Eurotium respectively, and produced, with few exceptions, on the aerial growth, demand conditions favorable for vegetative growth. The composition of the medium influences their production considerably, as in many media which produce good growths of mycelium they are wanting. The two structures do not always occur under the same conditions, although they usually do so. Their functions have not been determined.

(4) The fungus is very susceptible to the effects of alkalies and somewhat less so to acids. Of the three alkalies used, potassium hydroxide, sodium hydroxide, and sodium carbonate, the two former are the most injurious. Growth is retarded by acids the strength depending largely on the kind of acid and somewhat on the kind of medium in which it is used. Liquid media proved to be the most useful in determining the effect of acids and alkalies on growth. Acids of the acetic series were found to be the most injurious, growth usually refusing to take place in strengths above +6 1/4 in a liquid medium, while those of the hydroxy acid group are least so, development taking place in strengths +25 and sometimes stronger.

(5) Pigment production is retarded by weak solutions of alkalies and stronger solutions of acids. Weak solutions of some acids, i.e.,

malic, tartaric, citric, and lactic do under favorable conditions intensify the colors, such as salmon, pink, and red. The injurious effect on color production is more apparent in liquid than in solid media. High temperature, on the other hand, if not above 29° to 30°C favors the production of color, particularly the reddish purple in the substratum. Such a temperature does not retard growth but an increase to 35° to 37°C seriously retards and sometimes kills the fungus and prevents any formation of color. Color production is largely favored by light, the salmon color increasing rapidly in the aerial mycelium of cultures kept in the dark for a time and then submitted to the action of light.

(6) The pigment produced varies in color from salmon to purple, these two colors being most prominent. The production of the purple pigment was most abundant in rice tubes with a slightly alkaline reaction while the salmon occurred in almost all tubes. The behavior of these pigments to acids and alkalies and other substances lends probability to the supposition that there is a close relationship between the salmon and purple coloring matters, that they are very probably forms of the same compound, the former the acid and the latter the alkaline form. The production of color is retarded very little by osmotic pressure, according to the one experiment made.

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EXPLANATION OF PLATES

Plate I

Fig. 1.- From a germinating microconidium with its branching germ tube from a bouillon culture in a Van Tieghem cell.

Fig. 2.- From a terminally branched filament from a liquid culture made +3 1/8 with acetic acid.

Fig. 3.- A drawing of a terminally branched filament in a prune agar plate.

Fig. 4.- From another filament as Fig. 3.

Fig. 5.- A drawing showing the torulose nature of the submerged mycelium in a liquid culture +1 1/2 with oxalic acid.

Fig. 6.- A drawing showing a portion of a filament with a hypal branch producing microconidia and macroconidia. From an old prune juice culture in a growing cell.

Fig. 7.- From a microconidia producing hypha from a young prune juice culture.

Figs. 8-9.- From a branched conidia producing hypha and conidia from a prune agar plate.

Fig. 10.- A drawing of a much branched sporaphore from a bouillon culture.

Fig. 11.- Conidia from an infected ear of corn.

Plate II

Fig. 1.- From conidia germinating in distilled water and cutting off small microconidia.

Fig. 2.- From conidia produced and germinating in a tube of Uschinsky's fluid.

Fig. 3.- From Chamydosporoids grown in a cracked corn culture.

Fig. 4.- From coiled hyphae grown(a)and(b)in cracked corn,(c)in an acetic acid culture 12 1/2,(d)on rice, and(e)in a peptone glycerine solution.

Fig. 5.- From a segment of a filament enlarged by some substance, probably secreted, adhering to the outside. Produced on boiled parsnip.

Fig. 6.- From a swollen filament such as are produced in a solution containing 2% Witte's peptone, 1% dextrose, 1% maltose, and 1% mannite.

Fig. 7.- From a single hyphal branch in a 100% prune juice culture in a Van Tieghem cell.

Plate III

Fig. 1.- From a segment of a large filament giving off a branch of the smaller type.

Fig. 2.- (a) From a germinating conidium in an old prune juice culture in a growing cell, to which after drying down, a little water had been added. (b) From a filament in the same culture. The small portion and microconidia were produced after the addition of the water.

Fig. 3.- From macroconidia germinating in prune juice and originally taken from a diseased embryo ear of corn.

Fig. 4.- From a much branched sporaphore taken from a diseased embryo ear of corn.

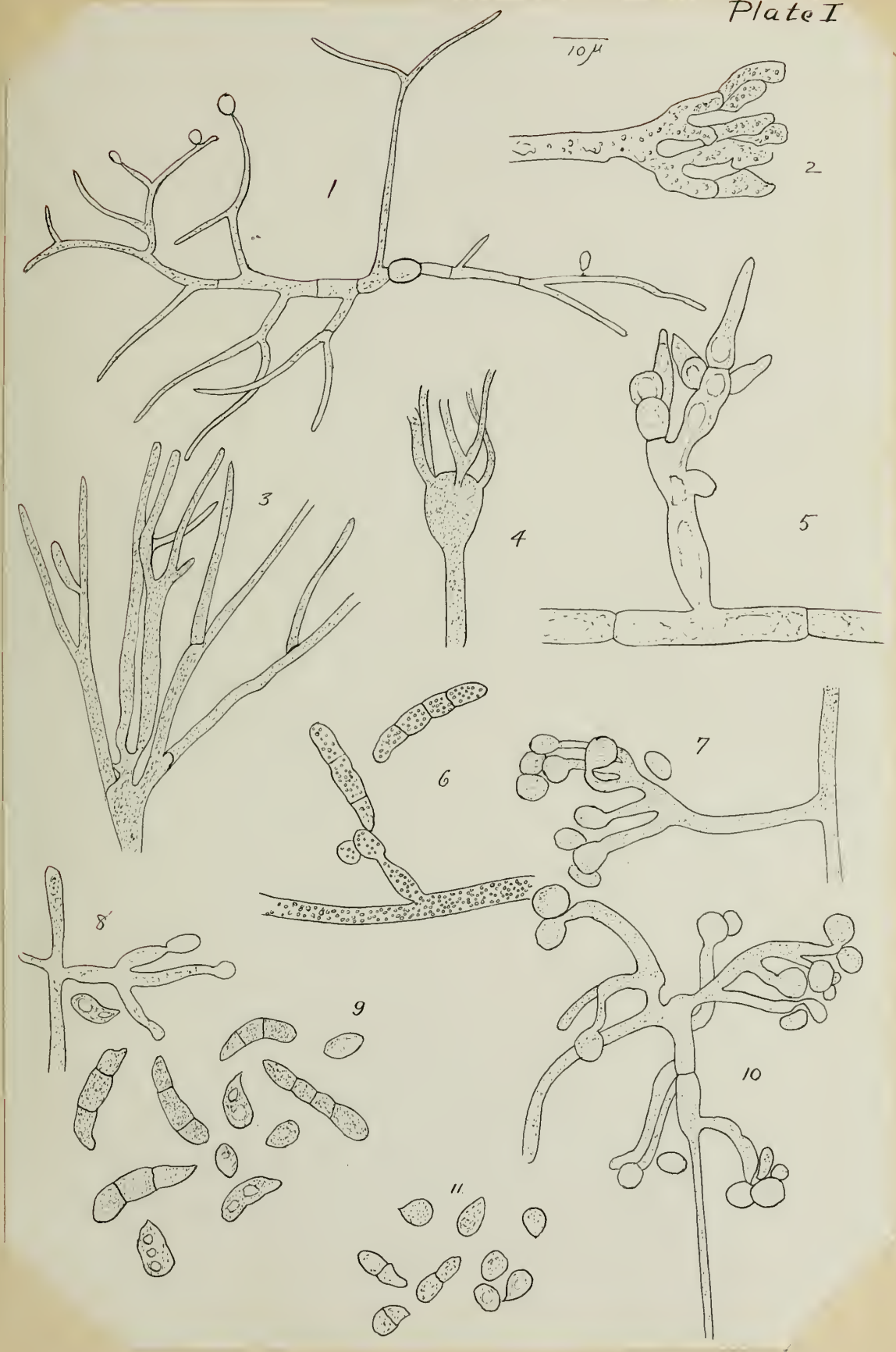
Fig. 5.- From macroconidia and microconidia taken from a diseased embryo of corn.

Plate IV

Fig. 1.- A photograph of the tubes used in temperature experiment No. I. (a) 23°C, (b) 30°C, and (d) 37°C.

Fig. 2.- A photograph of tubes used in temperature experiment No.3. (a) 23°C in light, (b) 13°C to 15°C in dark, (c) 23°C to 25°C in dark and (d) 30°C in dark.

Plate I



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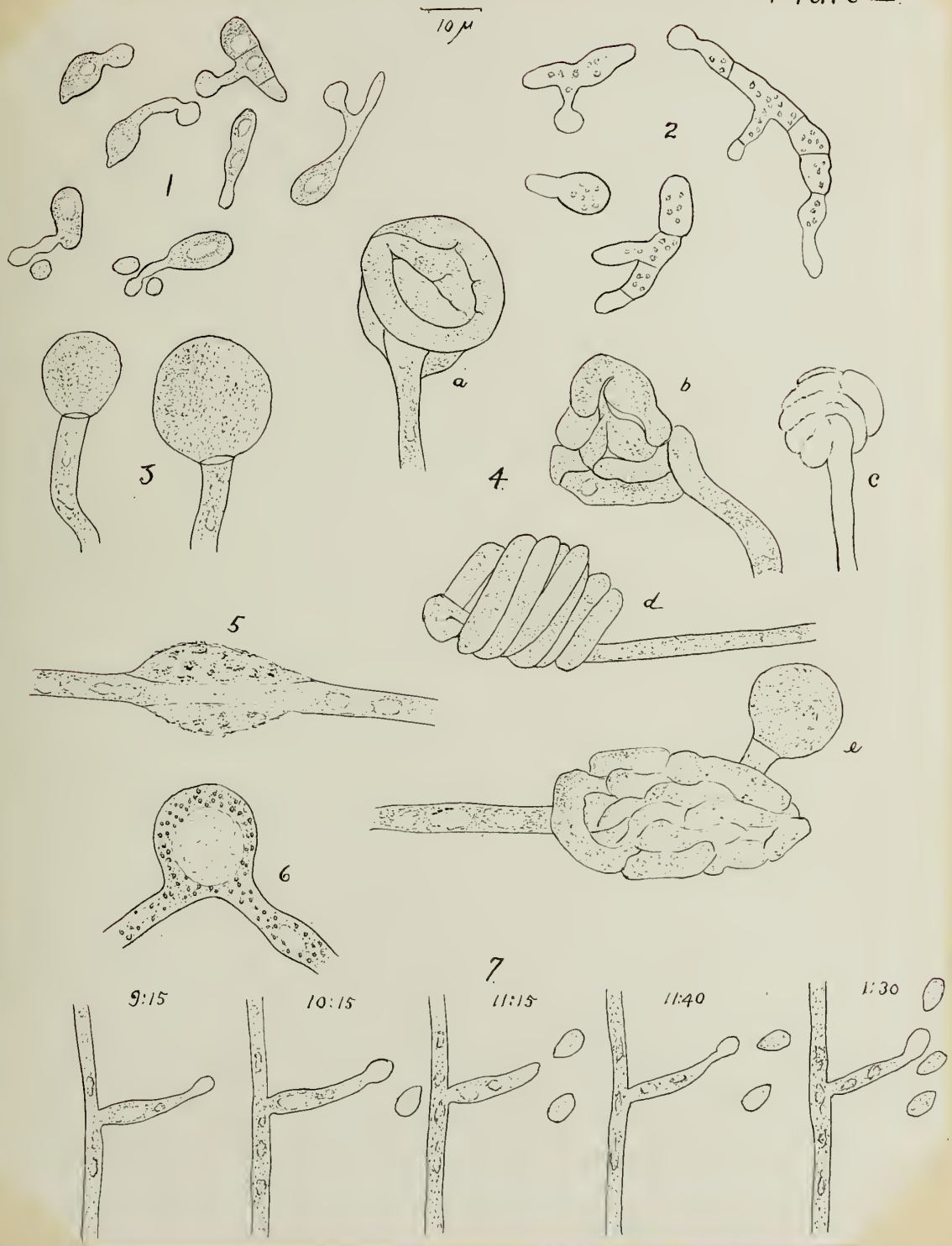
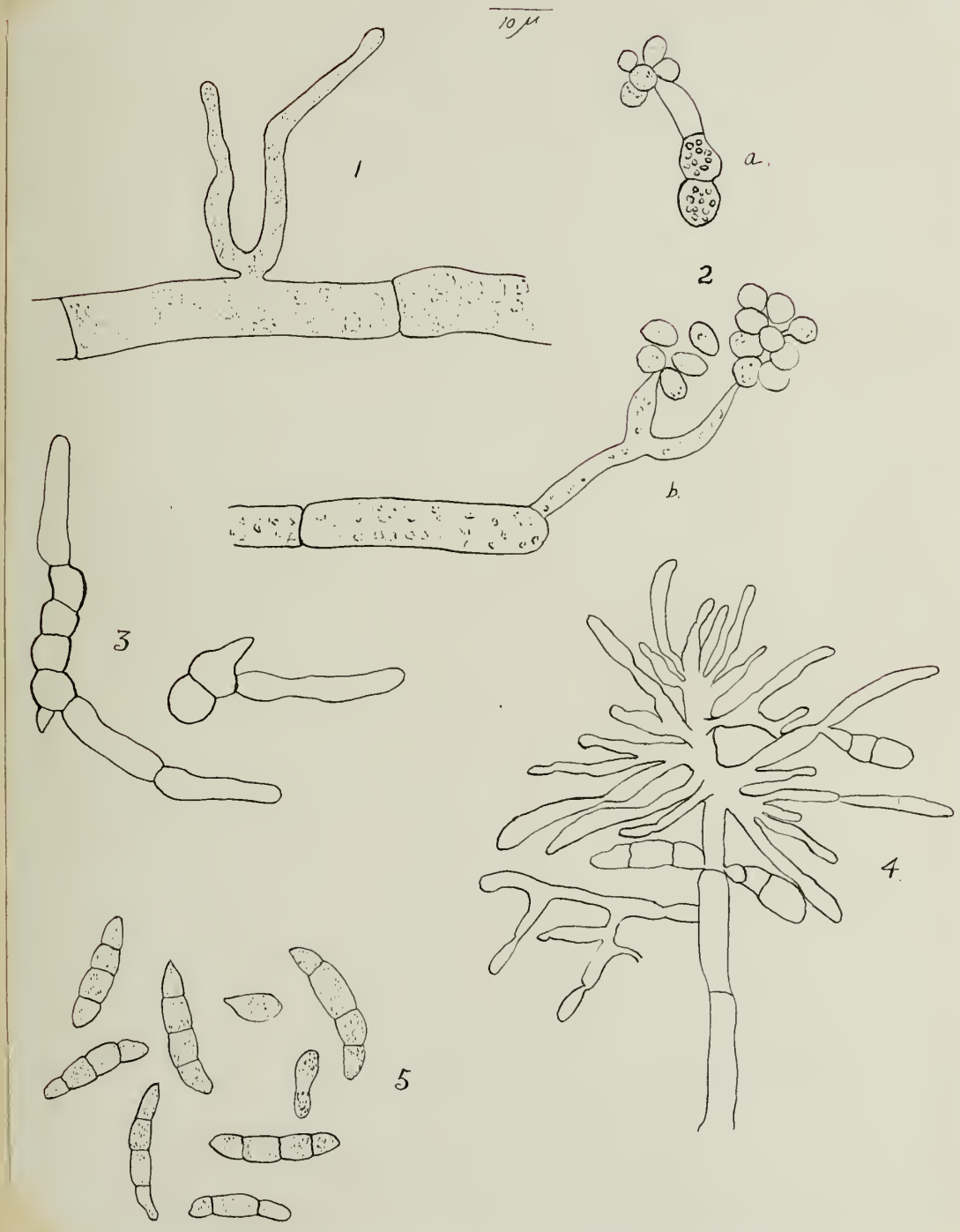


Plate III



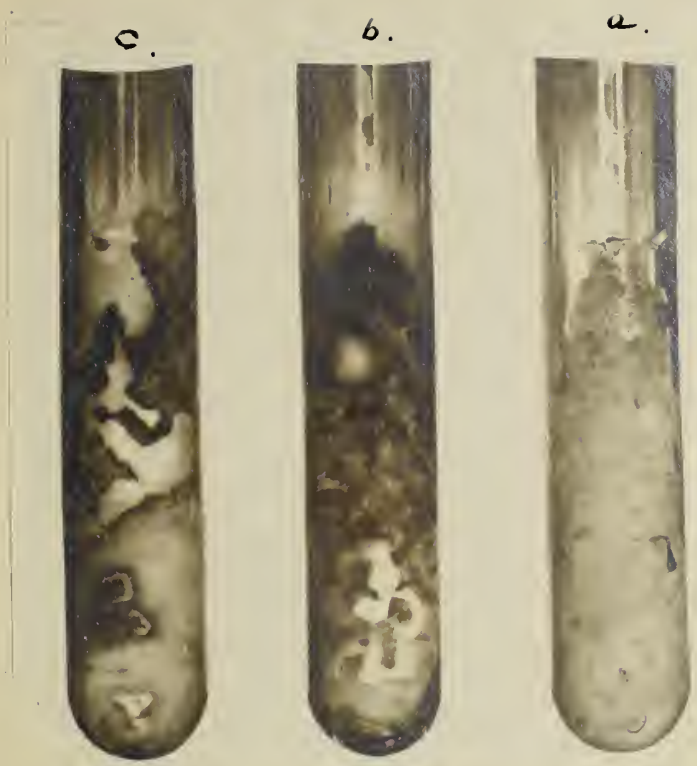


Fig. 1.

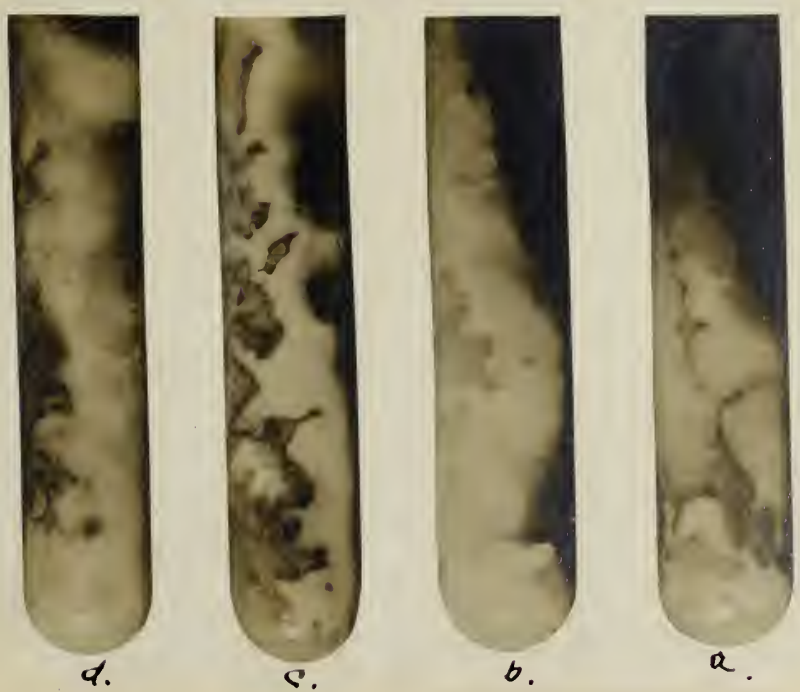


Fig. 2.